

Review article

The causes of the increasing prevalence of allergy: is atopy a microbial deprivation disorder?

Ö. Strannegård, I.-L. Strannegård

Departments of Clinical Virology and Pediatrics,
Faculty of Medicine, Göteborg University, Göteborg,
Sweden

Dr Ö. Strannegård
Departments of Clinical Virology and Pediatrics
Faculty of Medicine
Göteborg University
Göteborg
Sweden

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The atopic diseases, i.e., primarily, bronchial asthma, atopic dermatitis, and allergic rhinoconjunctivitis, were rare a few decades ago, but constitute today an increasingly severe public health problem. The increase in the prevalence of the allergic diseases is mainly seen in children (1). Recently, the “European Allergy White Paper” described the worrying increase in allergic diseases in Europe (2).

Formerly, allergic diseases such as hay fever were considered an exclusively upper-class disorder. In 1828, John Bostock described the hay fever symptoms in himself and in 27 other persons that he had found after meticulous scrutiny of all the clinics of London. In 1862, Philip Phoebus published a cogent monograph on hay fever based on data from 300 cases from the medical journals. He described the geographic as well as hereditary and social factors affecting his patients. In a book published in 1873 (3), Charles Blackley, himself a hay fever sufferer, provided clear evidence that pollen grains are the causative agents of the disease. He also claimed that catarrhus aestivus, also called hay fever or hay asthma, was more common among the educated than among the illiterate. He noted a curious circumstance regarding hay fever, namely, that the people who were most likely to be subjected to the action of pollen belonged to a social class which furnished the fewest cases of the disorder, i.e., agricultural laborers. Remarkably, Blackley not only made the interesting

observation of the association of hay fever and lifestyle but also predicted that “as civilisation and education advance, the disorder will become more common than it is at the present time”.

This is indeed what has happened. The increase in the prevalence of the allergic diseases, especially in those born after 1960, is almost explosive, and there are now epidemics of allergic diseases in many countries. The prevalence of asthma in children and young adults has tripled and quadrupled in many industrialized countries during the last two decades (1). It is often pointed out that the increase of the allergic diseases is associated with “westernization” of lifestyle. It is thus obvious that the atopic diseases correlate with socioeconomic levels. Generally, it seems clear that atopic diseases, especially hay fever, are more common among the wealthy than among the poor, in urban than in rural areas, and in the West than in the East (4–6).

Regarding the potential causes of the increase in the allergic diseases, it is important to remember that the induction of allergy, i.e., allergic sensitization, and the precipitation of allergic symptoms are two completely different phenomena. Therefore, it is theoretically possible that the same factor might *protect* against allergic sensitization, but also, if such sensitization has already occurred, *induce* allergic symptoms.

An analysis of the possible causes of the increased prevalence of allergy must be based on the assumption

that the occurrence of factors that have a potential capacity to stimulate allergic sensitization has increased or that hypothetically allergy-protective factors have been lost.

Pathogenic mechanisms

To understand how different environmental factors might influence the prevalence of allergy, we must elucidate the pathogenic mechanisms that are believed to underlie the allergic diseases. Atopic allergy is characteristically associated with an imbalance between various types of T cells and, as a consequence of this, an increased synthesis of IgE. Early studies revealed a T-cell imbalance that was found to be present in all the important types of childhood atopic diseases, such as bronchial asthma and hay fever, as well as atopic dermatitis (7). Later studies have shown that several subtypes occur among the T cells. Human T helper type 1 (Th1) cells are characterized by their ability to produce, among others, the cytokine IFN- γ . Th2 cells produce other cytokines, particularly IL-4, IL-5, and IL-13 (8).

This dichotomy is by no means absolute; there are other populations of T cells that release cytokines with other types of patterns, and many of the above-mentioned cytokines can also be produced by other types of cells. The common term "Th1/Th2 cell balance" is therefore somewhat misleading, and it may be preferable to talk about a balance between type 1 and 2 cytokines. Atopic diseases are characterized by an imbalance between these cytokines, so that type 2 cytokines are formed in abnormally high concentrations relative to the type 1 cytokines. This has been shown in many studies, for example, by stimulation of mononuclear cells from peripheral blood *in vitro* or by demonstration of cytokine-producing cells in tissues (9). Recently, we have shown that there is an allergy-associated imbalance between the amount of the type 1 cytokine IFN- γ and the type 2 cytokine IL-4 also in a body fluid, namely, nasal fluid from children and teenagers with hay fever (10, 11). An imbalance between IL-4 and IFN- γ may lead to an abnormally high production of IgE and of the various mediators that give rise to allergic symptoms.

In this context, it is important to point out that perhaps the most reliable predictive marker for atopic disease known today is a decreased ability of cord-blood lymphocytes to produce IFN- γ . A reduced ability to produce IFN- γ in the neonatal period has been shown to be closely correlated with an increased tendency to develop allergic sensitization and atopic diseases later in life (12–14).

As IL-4 and IFN- γ suppress the formation of each other (15), displacement of their delicate balance in either direction tends to be persistent, although there are other mechanisms that counteract this. An example of

this is the fact that histamine, which is produced during Th2-mediated allergic reactions, may stimulate the formation of IFN- γ (16).

Regarding the development of allergy, it is probably important that the intrauterine milieu is skewed toward the Th2 phenotype (17), and that allergic sensitization may occur already *in utero* (18). An imbalance between Th1 and Th2 cytokine production that occurs early in childhood might have an increased tendency to persist. Therefore, regarding the development of allergy, it is believed that the very first months of life are of crucial importance.

The genetic background of the atopic diseases is still not fully understood, but data hitherto presented show that both chromosome 5, where the gene for IL-4 is localized, and chromosome 12, which contains the gene for IFN- γ , may be involved (19). Even if many other genes seem to be of importance for the development of the atopic diseases, the genes for IL-4 and IFN- γ would be of particular interest since polymorphisms in these genes might be of immediate importance for the development of atopic diseases. Polymorphisms in the promoter region of the IL-4 gene, but not in the IFN- γ gene, have been described (20, 21). One study, however, has suggested that a candidate allergy gene is localized in a region of chromosome 12 that encompasses the IFN- γ gene (22).

Regarding possible factors that may influence the Th1/Th2 cytokine balance, it is noteworthy that prostaglandin E₂ (PGE₂) has a suppressive effect on IFN- γ secretion but does not, to the same extent, suppress the production of IL-4 or IL-5 (23). The effect of PGE₂ is probably mediated through cyclic adenosine monophosphate (cAMP). *In vitro* studies using phytohemagglutinin-stimulated peripheral blood mononuclear cells have shown that high concentrations of dibutyl cAMP may shift the IL-4/IFN- γ balance toward the Th2 side more than a hundredfold (our unpublished results). As the synthesis and release of PGE₂ is highly dependent on both diet and infectious agents, a search for potential environmental factors that might explain the increase of the allergic diseases should include factors that may stimulate PGE₂ activity or otherwise influence the Th1/Th2 cytokine balance.

If a given environmental factor is to be considered to be of importance in the increased prevalence of allergy, it should have the ability to polarize the cytokine balance toward Th2 immunity, and its occurrence in nature should explain the difference in the occurrence of allergic diseases between the rich and the poor, between urban and rural areas, and between the East and the West. We will now discuss some environmental factors that might be responsible for the increased prevalence of the allergic diseases and their relation to the above-mentioned criteria.

Air pollution

It is a common belief, particularly among the adherents of various environmental pressure groups, that the increase of the allergic diseases has occurred because of the increased air pollution emanating from traffic and various industries. However, there are no scientific data to support this belief. In fact, recently published results from the global study of asthma and allergies in childhood – the ISAAC study (1) – indicate that air pollutants seem to have no causal connection with the increased occurrence of allergic diseases. For example, the prevalence of allergic diseases is very high in New Zealand, where the air is very clean and clear. Allergic diseases are also more common in Western than Eastern Europe, although air pollution undoubtedly is much greater in the East than in the West.

It has been proposed that a relative loss of some environmental protective factor or factors that were common in preindustrial times could explain the increase in the prevalence of the allergic diseases. It is indeed possible that certain types of air pollutants may protect against the development of allergic diseases. For instance, heavy exposure to swine dust results in a marked release of cytokines that may stimulate Th1 immune responses and therefore secondarily suppress Th2-type immunity (24). Chronic environmental exposure of young children to house-dust endotoxins, derived from the cell walls of Gram-negative bacteria, has recently been found to be associated with protection against allergy in young children (25, 26). Although it was proposed that this may be due to the capacity of endotoxin to stimulate Th1 immunity, it seems equally possible that endotoxin is merely a marker of the bacterial content of the house dust, and that some other components of the environment are the actual allergy-protective factors. For instance, it has been demonstrated that Gram-positive bacteria are much more potent inducers of the Th1 immunity-associated cytokine IL-12 than are the endotoxin-containing Gram-negative bacteria (27). Regardless of the nature of the putative factors protecting against allergy, it seems plausible that various types of bacteria, and glucans or spores from molds, which occur as air pollutants, may actually displace the Th1/Th2 cytokine balance and thus protect against the development of allergic sensitization.

In this context, it is of particular interest that several recently performed studies have unequivocally shown that children who are raised on farms are less likely to develop atopic allergy and allergic sensitization to allergens such as grass pollen and tree pollen than children raised in other environments (28–31). In particular, the lower prevalence of allergic diseases has been found to be dependent on close contact with cows and other farm animals. Taken together, these results strongly suggest that an environment characterized by a high content of bacteria and other particulate matter in

the air may indeed protect against development of allergy, at least if an individual is subjected to such an environment during infancy.

Although it may be assumed that a general Th1-stimulating ability of microbial contaminants may be responsible for the apparent allergy-protecting effect of polluted air, this is by no means clear. Although the effect seems to be nonspecific, it cannot be excluded that specific immunologic tolerance plays a role in protection. In other words, polluted air may contain bacterial products that stimulate Th1-type immunity, but also excessive amounts of various antigens, such as mites and pollen grains, that may evoke specific immunologic tolerance.

It is often claimed that a bad indoor climate could contribute to the increase of the allergic diseases. In support of this hypothesis, it is pointed out that houses built during the last few decades are airtight and often poorly ventilated. Therefore, the indoor air contains many allergens and other potentially allergy-triggering substances, as well as a high level of humidity, providing optimal conditions for the growth of mites. Several studies have indeed documented a positive correlation between allergy prevalence and bad indoor climate (32). However, this association may simply be due to the fact that excessive presence of allergens in the indoor air is likely to precipitate allergic symptoms in an *already sensitized individual*. Thus, these findings do not provide any evidence that a poor indoor climate *per se* plays any causal role in the increasing allergy prevalence.

In fact, it seems highly unlikely that the global increase of the allergic diseases in childhood should depend on a worsening indoor climate. Some years ago, when allergies were infrequent, people often lived in damp houses with an abundance of aeroallergens, such as animal epithelia and pollen grains, especially in the countryside. Allergic diseases have increased in many countries where the homes are both dry and very well ventilated. In a study from Kuwait, a desert country with an extremely dry and hot climate, we were not able to find any reason to assume that the indoor climate was responsible for the rapid increase of allergic diseases in this country (33).

Diesel exhaust particles

In the context of air pollution, automobile exhaust particles are of particular concern. The increase of the allergic diseases has occurred in parallel with the increased use of fossil fuels. Air pollutants emanating from these, especially diesel exhaust particles, have been found to stimulate the formation of IgE both *in vitro* and *in vivo* (34). Diesel particles have also been shown to stimulate the gene expression and the formation of Th2 cytokines, as in nasal secretions (35, 36).

Even if, from a theoretical standpoint, diesel exhaust

particles may be suspected of being causally related to the increasing allergy problem, no convincing epidemiologic data have established that this actually is the case. In a study from Japan a higher prevalence of allergy to cedar trees was found in people living close to a highway than in persons who were exposed to the same amounts of pollen, but who were not to the same extent exposed to automobile exhaust (37). These results have, however, been challenged by other investigators who failed to find any relationships between traffic exposure and the prevalence of hay fever or asthma (38).

Tobacco smoke

Among the adjuvant factors that have been proposed to be responsible for the increase of allergic diseases, tobacco smoke is of particular importance. Early studies have shown that tobacco smoke administered to rats enhances the formation of IgE antibodies (39). Later studies have indicated that this effect probably can be explained by the stimulatory effect of smoke on IL-4 synthesis. Children who are exposed to environmental tobacco smoke have been found to have eosinophilia, and increased serum IgE and IL-4 levels, together with an increased tendency to develop frequent respiratory symptoms (40, 41). Passive smoking during or after pregnancy has been shown to be a risk factor for the development of both allergic sensitization and obstructive respiratory disease in the child (42).

Even if smoking has a proven effect on allergic diseases, it does not seem probable that it is a major causative factor in the global increase of these diseases. Thus, differences in the use of tobacco between different socioeconomic groups or between different geographic regions are by no means paralleled by differences in allergy prevalence.

Net effects of air pollution factors

The discrepant results of studies on factors such as the effects of traffic exposure on the prevalence of allergy should be viewed in the light of the recent findings that certain pollutants, such as bacterial products, possibly prevent, whereas others, such as diesel exhaust particles and tobacco smoke, may enhance the development of allergy. Thus, comparative studies of allergy prevalence yield results that depend on the net effects of allergy-promoting and -preventing factors.

Taken together, the results of large epidemiologic studies provide convincing evidence that increased air pollution in general is not responsible for the increasing prevalence of allergy. Rather, it seems more likely that decreased air pollution, such as that by bacterial products, may be causally related to the increasing prevalence.

New allergens

Introduction of new allergenic chemical compounds, or "new allergens", from the animal or vegetable kingdom is often suspected of causing the increase of allergic diseases. It should be emphasized, however, that the increase of allergic diseases mainly concerns specific sensitization to well-known "natural" allergens such as animal dander; pollen from weeds, grass, or trees; house-dust mites; and various foodstuffs such as eggs, milk, and fish, and that the occurrence of these, with very few exceptions, has not increased during the last decades.

In some cases, however, new allergens have been claimed to be of importance in this regard. For instance, the introduction in Kuwait of the quickly growing tree *Prosopis juliflora*, whose pollen is a potent allergen, coincided with the increase of the prevalence of the allergic diseases (43). In New Guinea, house-dust mites were introduced through import of contaminated blankets, and in the ensuing years the prevalence of asthma increased more than 20-fold (44).

The often cherished thought that cleaner air and fewer chemicals in the environment would lead to an allergy-free community has no solid scientific basis. However, it is important to realize that this by no means implies that allergen avoidance is unnecessary. A person who is already sensitized to allergens will always be at risk of developing allergic symptoms when living in environments where allergens are prevalent. In other words, although secondary allergen prevention is necessary in the battle against allergies, it seems less probable that allergen avoidance could be effective in primary allergy prevention.

Hormones and environmental poisons

Increased stress in the community has been proposed to be one of the predisposing factors for the development of allergy. Stress may modulate the immune system and change the balance between Th1 and Th2 cytokines toward Th2 immunity. This effect could possibly be mediated through glucocorticosteroids, as dexamethazone may stimulate the production of Th2 cytokines *in vitro* (45) and induce changes in human cytokine patterns that are considered to be stress-related (46). Similarly, catecholamines that are released during stress reactions may raise intracellular levels of cAMP and thereby evoke Th2 polarization (Strannegård & Strannegård, unpublished results).

Female sex hormones occur in increasing amounts in nature in the Western world due to the increased use of contraceptive pills. It is of interest to note that the altered relation between sex hormones that occurs in the pregnant woman contributes to the skewing of the immunity toward the Th2 type of immunity, which is typical of pregnancy (17). It is well established that

progesterone favors Th2-type immunity (47), and increased occurrence of this hormone in nature might therefore enhance development of allergy.

In many countries an increase of environmental toxins such as DDT and PCB in nature has coincided with the increase in the prevalence of atopic diseases. Speculation on a possible causal relationship between toxic products in the environment and allergy has been based on epidemiologic data, but a possible mechanism for their action has, to our knowledge, not been presented. However, the results of a study by Svensson et al. (48) may explain the suggested relationship. This study showed that the number of CD56-positive natural killer (NK) cells was depressed in people who consumed large amounts of salmon from the Baltic Sea, which is known to contain large amounts of environmental toxins. The number of NK cells in this population was shown to be inversely related to the serum levels of DDT and PCB. NK cells are an important source of IFN- γ ; consequently, a depression of the amount of circulating NK cells should be assumed to be accompanied by a decreased production of IFN- γ and thereby a higher IL-4/IFN- γ ratio, which, in turn, should predispose to the development of allergy.

Although several of the factors mentioned above potentially evoke Th2 polarization, there are no epidemiologic data that make it likely that any of the factors have a major effect on the global prevalence of allergy.

Reduced frequency of bacterial and viral infections

Several studies have shown that children with many siblings have a reduced frequency of allergic diseases (49, 50). The inverse relationship between the number of siblings and allergy is particularly evident regarding the number of brothers. The more children in the family, the more infections they encounter. This has led to the hypothesis that a high load of infections during early childhood may help to prevent allergy. As most infections during childhood are caused by viruses, the interest has been focused on viral infections. For instance, it has been pointed out that in some geographically isolated areas, such as the Western Caroline Islands and Tristan da Cunha, the prevalence of allergic diseases is very high, whereas the prevalence of infections caused by ordinary respiratory viruses is low (51). Further evidence that early viral infections might be protective has been obtained for measles (52) and hepatitis A (53). The hepatitis A virus is closely related to the enteroviruses, which include more than 70 different serotypes. Enteroviruses are potent inducers of IFN- γ (54), and these viruses are particularly prevalent in developing countries, where allergic diseases generally occur less frequently than in developed countries.

The hypothesis that infections in general could have a

protective effect against the development of allergic diseases is contradicted by data that suggest that some viral infections, most notably those caused by the respiratory syncytial virus (RSV), might stimulate the development of allergic sensitization as well as asthma (55, 56). Another virus, the Epstein-Barr virus (EBV), has been shown to have a special ability to induce an IgE response; thus, this virus may be suspected of having a stimulatory rather than dampening effect on the development of allergy. Studies of children and adults with atopic diseases, have demonstrated increased antibody titers to EBV (57, 58). Altogether, the results regarding viral diseases suggest that, although some viruses may be able to stimulate the development of allergic diseases, most viral infections seem to be protective.

Viral infections generally induce a strong, cell-mediated immune response; i.e., an immune response that is mainly driven by Th1 cells and IFN- γ . Intracellular bacteria such as mycobacteria have the same ability; therefore, it has been assumed that early infections with, in particular, *Mycobacterium tuberculosis* could protect against the development of allergic diseases later in life. A study reported by Shirakawa et al. (59) supports this assumption. However, the conclusions of that study were based on an interpretation that failed to take into account that individuals with an atopic constitution have an impaired Th1 immunity (60). This leads to a depressed reactivity in the tuberculin tests, and the cause-effect relationship between absent tuberculin reactivity and atopy may therefore be the opposite of that claimed by Shirakawa et al. In later studies on the reactivity to tuberculin and sensitins from atypical mycobacteria in more than 6000 children, we did not find any evidence that early infections with mycobacteria protect against the development of allergic diseases later in life (61). This finding has been confirmed by others (62). It should be noted, however, that results based on testing for delayed hypersensitivity will not allow firm conclusions regarding the relationship between atopy and mycobacterial infections for the reason given above.

If the assumption that early viral or bacterial infections protect against the development of allergic diseases is correct, vaccination should lead to an increase of allergic disorders. This hypothesis may be supported by a study by Alm et al., who showed that Swedish children raised in an anthroposophic milieu had a significantly lower prevalence of allergic diseases than children raised in other environments (63). This study found that the prevalence of atopy was positively correlated to MMR vaccination (measles, mumps, rubella) and with the administration of antibiotics. There are conflicting reports on the association of pertussis vaccination with development of allergy (64, 65). Likewise, the effect of BCG vaccination on the development of atopy remains controversial; two studies from Sweden failed to find any protective effect of BCG immunization (61, 66), whereas there was a strong allergy-protective effect in a study

from Guinea-Bissau (67). To sum up the available data, we may say that there is still no convincing evidence that childhood vaccinations have any major impact on the development of atopic disease. This is perhaps not surprising because children are immunized against only a very few of the putatively allergy-protective viruses and bacteria that infect children.

Regarding antibiotic treatment, the situation is different, since antibiotics essentially act nonspecifically and have the ability to kill not only pathogenic but also commensal bacteria. Studies by Farooqi & Hopkin (64) revealed a significant relationship between treatment with antibiotics during the first 2 years of life and later development of allergy. Particularly convincing are the dose-response relationships; i.e., the finding that multiple courses of antibiotic treatment are associated with higher allergy prevalence, and the finding that treatment with broad-spectrum antibiotics appears to be more likely to be associated with allergy development than is ordinary penicillin. The results of Farooqi & Hopkin have been corroborated by results obtained in a study from New Zealand (68). In Sweden, a study showed a striking parallel between prescription of antibiotics and the increasing prevalence of allergic rhinitis over the last decades (Sten Lindgren, personal communication).

Taken together, the results presented above suggest that microbial agents do indeed play a protective role in the development of allergic disease. Further support for an allergy-protective ability of microbial agents could be obtained from studies mentioned above (28–31), which clearly imply a protective effect of the lifestyle of farmers and their children. A study from Germany showed that children from small (but not from large) families developed allergic sensitization with a frequency that was directly correlated to the age at which they began to attend day-care centers (69). Thus, allergic sensitization was about twice as common in the children who had started at the age of 1–2 years as in children who were admitted before 1 year of age, and was almost tripled in the children who started at the day-care center at the age of 2 years or more. It is hard to find any other explanation for these findings than that the exchange of infections among the children was the cause of the lower allergy prevalence. This interpretation agrees with that usually offered to explain the well-established fact that the risk of developing allergy is inversely correlated to the number of siblings (70). However, the nature of the putatively allergy-protecting infections is obscure. Given the seemingly strong effect of early antibiotic treatment mentioned above (64, 68) and the potent effects of certain bacteria on the Th1/Th2 cytokine balance (27), it is tempting to assume that the protective effect may be primarily evoked by bacterial infections.

From an evolutionary perspective, it is perhaps not unexpected that the immune system, which over millions of years has adapted to a heavy microbial load, may react in an “inadequate” way upon a sudden, radical decrease

of this load, caused by vaccinations, antibiotics, and especially improved hygienic conditions. The disappearance of certain viral diseases that earlier were very common among young children (e.g., polio and measles) and a drastic reduction of the number of severe bacterial infections (e.g., tuberculosis) could be of particular importance in this regard. The loss of these putatively protective factors might alter the normal maturation of the immune system from the Th2 type of immunity, which is considered to prevail during pregnancy and in the neonate (17), to the Th1/Th2 balance that occurs in adults. These circumstances add to the probability that a changed spectrum of infections could be an important cause of the increased allergy prevalence.

In view of the vast number of different agents that can infect man, we should not expect that a change in the prevalence of some or a few viruses or bacteria would have any major impact on the prevalence of the atopic diseases, even if a change in the spectrum of infections alone were responsible for the increase. Thus, it may seem astonishing that an inverse relationship between the prevalence of atopy and a single virus such as that of hepatitis A has been observed (53). However, infection by the hepatitis A virus is a strong marker of poor hygiene (71), and since the association between poor hygiene and protection against atopy is very clear (6), any infection whose prevalence is dependent on hygienic standards would be expected to show an inverse association with allergy. In agreement with this prediction, a recent report by Matricardi et al. showed an inverse relationship between respiratory allergy and past infection with the hepatitis A virus, *Helicobacter pylori*, and *Toxoplasma gondii*, but no relationship between allergy and rubella, mumps, measles, chickenpox, cytomegalovirus, or herpes simplex virus infection (72).

Altered gut flora

Allergies can be regarded as manifestations of aggressive immune responses to antigens that are usually well tolerated by the host. Substances introduced perorally are normally ignored by the immune system, a phenomenon called oral tolerance. Several mechanisms, including T-cell depletion, T-cell anergy, and induction of regulatory T cells, seem to be involved in the development of oral tolerance. Activated regulatory T cells secrete inhibitory cytokines, particularly IL-10 and TGF- β (73). In order for oral tolerance to be induced, a normal microflora in the gut would seem to be mandatory. Thus, animals raised in a sterile, germ-free environment will not develop normal tolerance (74). This has been claimed to depend on the lack of lipopolysaccharide (LPS), which seems to be necessary for the normal development of oral tolerance (75). LPS is a constituent of the external part of the cell membrane of Gram-negative bacteria.

Formerly, healthy neonates were rapidly colonized by

E. coli or other enterobacteria during their first days of life, but in countries characterized by a Western lifestyle the colonization seems to occur much more slowly (76). The spread of *E. coli* can be considered a sign of poor hygienic conditions, and with an improved hygienic level fewer children will show early colonization with these bacteria.

Delayed colonization by Gram-negative bacteria leads to diminished levels of LPS in the gut; thus, oral tolerance may not develop normally. If the children do not encounter enterobacteria, they will be colonized by other bacteria. Early colonization by *Staphylococcus aureus* seems to be increasingly common in developed countries (76).

An association between the gut flora and the development of allergy has been based on findings of differences in the gut flora between allergic and nonallergic children (77, 78). Lactobacilli may be of particular interest in this context since they are known to be potent inducers of IL-12, which is a key cytokine in Th1-type immunity (79). Lactobacilli have been claimed to occur more frequently in nonallergic than in allergic children, and they have also been claimed to be of therapeutic benefit in children suffering from severe atopic dermatitis (80). Recently, it has been shown that the capacity of lactobacilli to elicit production of IL-12 is equaled by other Gram-positive (but not by Gram-negative) bacteria (27); thus, there is no reason to assume that lactobacilli are uniquely important for protection against allergy. It is, however, of interest to note that the anthroposophic lifestyle includes a diet including acidified vegetables, which contain lactobacilli, and, as already mentioned, children from an anthroposophic environment have fewer allergies than other children (63).

Although interesting results have been obtained in the studies referred to above, interpretations of associations between the gut microflora and allergy obtained in cross-sectional studies should be made with caution. It seems clear that the hygienic standard correlates with allergy, as well as with the type of intestinal microflora. It should, then, come as no surprise if the latter two factors show some degree of correlation. More solid data will be derived from ongoing prospective studies of the relation of the microflora to the development of allergy.

In the context of the possible influence of bacteria on the Th1/Th2 system, it is of special interest to mention that bacterial DNA, containing certain so-called immunostimulatory nucleotide sequences, has been shown to be able to stimulate Th1 immunity preferentially (81, 82). "Hyposensitization" through immunization with plasmid DNA composed of genes for allergens in combination with immunostimulatory sequences is currently being tested in animal experiments with the aim of stimulating Th1 immunity (83).

If, because of changed hygienic conditions, the normal bacterial colonization is altered, it seems plausible that both the induction of tolerance and allergy-dampening

Th1 immunity might be affected by the above-mentioned mechanisms. An altered bacterial colonization, especially in the very young baby, might therefore lead to a persistently predominant Th2 type of immunity and later to the development of allergic diseases.

Changes in consumption of dietary fatty acids

Atopic allergy is more common in rich than in poor countries. Malnourishment does not predispose to allergy, although it has a suppressive effect on the immune response. It is, however, of interest to note that deficiency of vitamin A, which is often associated with malnutrition, in animal experiments has been shown to lead to an increased production of IFN- γ as a part of a generally increased inflammatory response (84). This finding might partly explain why atopic allergy is rare among malnourished children.

It has been known for many years that atopic individuals have abnormal proportions of various polyunsaturated fatty acids in their blood. In a study of children with atopic dermatitis, we found increased levels of linoleic acid and decreased levels of arachidonic acid, as well as a correlation between levels of IgE and linoleic acid in cord blood (85). Black & Sharpe (86) have pointed out that the relative linoleic acid content in fatty tissues from the normal population in Western countries has increased in parallel with the increasing prevalence of asthma during the last decades. They also showed that there is a correlation between the reported prevalence of allergic rhinitis in different regions in Finland and the ratio between the plasma concentrations of omega-6 and omega-3 fatty acids.

These results support the hypothesis that the increase of the allergic diseases is dependent on a change of eating habits (86). In Western countries, there has been an increase in the consumption of linoleic acid-containing food, such as margarine, whereas the consumption of omega-3 fatty acids that are present in high amounts, as in fish oil, has diminished. The practice of giving small children fish oil to provide them with fat-soluble vitamins has largely been abandoned. In many countries, the consumption of fish containing large amounts of omega-3-polyunsaturated fatty acids has decreased. Recently reported results show that the difference in the prevalence of allergic diseases between East and West Germany corresponds to a difference in the consumption of margarine (87). Thus, both the prevalence of allergic diseases and the consumption of margarine (instead of butter) was much higher in West Germany at the time of the study. A similar relationship between consumption of margarine and prevalence of allergic diseases has been reported by others (88, 89).

The theoretic background for the hypothesis of Black & Sharpe is the following. Linoleic acid is a precursor of arachidonic acid, which, in turn, is a precursor of

prostaglandin E₂ (PGE₂). Long-chain fatty acids from the omega-3 series compete with omega-6 fatty acids regarding several different enzymes that lead to the formation of PGE₂, an effect which therefore will occur in reduced amounts in the presence of omega-3 fatty acids. As mentioned above, PGE₂ has a strong inhibitory effect on the formation of IFN- γ but a tendency to increase the formation of IL-4 (23). A change in diet to a higher intake of omega-6 and a reduced intake of omega-3 fatty acids should therefore lead to an increased activity of PGE₂ and a polarization toward Th-2 type immunity.

The neonate has an immature immune system influenced by the intrauterine skewing of immunity toward the Th2 type (17). Breast milk is considered important for the further development of the baby's immune system. Therefore, it is of interest that the breast milk of atopic mothers appears to have a higher linoleic acid/ α -linolenic acid (i.e., omega-6/omega-3) ratio than milk of nonatopic mothers (90). Moreover, the cytokines and chemokines in breast milk differ between atopic and nonatopic mothers. Thus, atopic mothers have been found to have higher levels of IL-4, IL-5, and IL-13 in their colostrum than nonatopic mothers; similarly, increased levels of RANTES and IL-8 have been found in the milk of allergic mothers (91).

Because of the different composition of the breast milk of atopic and nonatopic mothers, it seems possible that children who have inherited their atopic predisposition from the mother will not benefit from the possible allergy-protecting effect of the breast milk, whereas, by contrast, children who inherit the allergy from their fathers might be protected. This possibility might at least partly explain the discrepant findings on the protective effect of breast-feeding against the development of allergy.

Regarding the importance of fish oils, it is interesting to note that the prevalence of asthma is low among the Inuit, who have a high intake of fish oils (92). An Australian study found that children who ate fatty fish such as salmon, tuna, and sardines had a lower risk of developing asthma than children eating other types of food (93). Another study showed that Asian school-children who kept to their Asian diet had a lower prevalence of asthma, hyperreactivity and atopy than non-Asian children or Asian children who had adopted Western dietary habits (94). The hypothesis that changed feeding habits could be of importance for the increased prevalence of allergies is thus supported both by epidemiologic data and the fact that there is a plausible mechanism of action for the effect.

The possibility that a difference in the prevalence of allergic diseases could be dependent on several cofactors has been explored in interesting animal experiments (95). In these experiments, rats were fed either a fish oil (containing omega-3 fatty acids) or a control oil (containing omega-6 fatty acids). After infection with *Listeria monocytogenes*, the rats that had been fed fish oil

developed significantly higher levels of IFN- γ in the blood than the control animals. This finding would be consistent with the assumption that fish oil decreases the production of the IFN- γ -suppressive agent PGE₂.

Changes in dietary antioxidants

Free oxygen radicals, which are formed during various inflammatory processes, have a suppressive effect on certain types of immune responses. For instance, activated monocytes/macrophages form free radicals, such as hydrogen peroxide, which particularly suppress the activity of NK cells but also affect T cells (96). This effect may be abrogated by histamine, which suppresses the formation of hydrogen peroxide. A similar effect is also exerted by serotonin and antioxidants such as catalase (16). The net result of treatment with antioxidants might therefore be that the synthesis of IFN- γ is increased, whereas the synthesis of IL-4 is not affected, since the NK cells do not produce this cytokine. In recent studies, we have found that the secretion of IFN- γ by phytohemagglutinin-stimulated mononuclear cells is more easily suppressed by hydrogen peroxide than is the secretion of IL-4 (Strannegård & Strannegård, unpublished results). It thus seems clear that hydrogen peroxide, which is an important reactive oxygen species, has a capacity to polarize the immune system toward Th2 immunity.

The fact that free oxygen radicals have a strongly inhibitory effect on the production of IFN- γ suggests that antioxidants in the diet may have an antiallergic effect. This hypothesis is supported by the finding that the blood concentration of glutathione peroxidase, an important antioxidant, is abnormally low in both adults and children with asthma (97) and by the finding of an inverse relationship between intake or plasma levels of natural oxidants and adult-onset wheezing (98). Furthermore, a low intake of vitamin C and manganese has been associated with bronchial hyperreactivity (99), and, most importantly, polymorphisms at the glutathione S-transferase GSTP1 locus have been linked to the risk of developing atopy (100).

Taken together, the above results suggest that oxidative stress is important, not only for the pathogenesis of airway inflammation and asthma, but, possibly, also for allergic sensitization. The continually decreasing intake of dietary oxidants that has been observed in developed countries over the past 30 years (101) may therefore have contributed to the rising allergy prevalence.

Acetylsalicylic acid and young children

According to a recently published hypothesis (102), the increased allergy prevalence could be partly explained by a continually diminishing use of acetylsalicylic acid in young children. This has been occurring since the begin-

Table 1. Environmental factors that may be supposed to be causally related to increasing allergy prevalence. Factors are listed in suggested order of probability of causality

Factor	Polarization of Th1/Th2 balance toward Th2	Epidemiologic criteria fulfilled	Possible causative factor
Low frequency of Th1-stimulating infections due to improved hygiene, antibiotics, vaccinations	Yes; decreased synthesis of IFN- γ	Yes	Yes
Changed intestinal bacterial flora (particularly improved hygiene)	Yes; some bacteria may induce oral tolerance, and some stimulate Th1 immunity	Yes	Yes
Decreased exposure to airborne bacteria	Yes; less stimulation of IFN- γ and IL-12	Yes	Yes
Changed dietary habits: less omega-3 and more omega-6 fatty acids	Yes; PGE ₂ increases IL-4/IFN- γ ratio	Yes	Yes?
Changed dietary habits: less antioxidants	Yes; reactive oxygen species may increase IL-4/IFN- γ ratio	Yes	Yes?
"New allergens"	Yes; but only in allergen-specific way	Not generally	Yes?
Poor indoor climate	Yes; indirectly via tobacco smoke and "new" allergens	Partly	Yes?
Diesel exhaust	Yes; increased synthesis of IL-4	Partly	Yes?
Tobacco smoke	Yes; increased synthesis of IL-4	No	Yes?
Stress, hormones	Yes; may increase IL-4/IFN- γ ratio	Partly	No?
Environmental poisons (e.g., PCB, DDT)	Yes; decrease of NK cells leads to decreased synthesis of IFN- γ	No	No?
Less acetylsalicylic acid to small children	Yes; PGE ₂ increases IL-4/IFN- γ ratio	Partly	No
General air pollution	No	No	No

ning of 1980 because of the recognition of a relationship between intake of acetylsalicylic acid and development of Reye's syndrome. The proposed mechanism for the effect is the following. In upper respiratory tract viral infections, several products of the arachidonic acid cascade are induced, particularly after the initial release of cytokines (103). Proinflammatory cytokines, such as IL-1, activate the enzyme cyclooxygenase 2 (COX-2) that promotes the formation of PGE₂. As described above, PGE₂ tends to polarize the Th1/Th2 cytokine balance toward Th2 dominance. Acetylsalicylic acid blocks the production of COX-2 and therefore decreases the production of PGE₂. Other pyretics, such as paracetamol, have other modes of action. The replacement of acetylsalicylic acid with paracetamol in febrile children therefore allows the immunomodulatory effects of PGE₂ to occur, and thus promotes the development of asthma and allergies.

In the USA, the increased use of paracetamol in children has paralleled the increase of the prevalence of allergy (102). Generally, however, the available epidemiologic data do not suggest that the global increase of the allergic diseases may be explained by the decreased use of acetylsalicylic acid.

Comparison between candidate causative factors

With the criteria used in this review as a basis, several environmental factors may be suspected of contributing to the increase in the prevalence of the atopic diseases. Of the factors that are mentioned in Table 1, a diminished microbial load, mainly caused by improvement of hygienic conditions, may be assumed to be the most important factor. Improved hygiene is associated with a decreased frequency of Th1 immunity-stimulating infections, as well as with changes in the commensal bacterial flora. Another possible cause of the rising allergy prevalence could be changed dietary habits that include alteration of the proportions between omega-6 and omega-3 fatty acids and decreased intake of antioxidants, both of which may lead to Th2 polarization of the immune system. Several other environmental factors have a similar effect on the Th1/Th2 balance, and changes in the occurrence of these factors coincide in several cases with the increase of the allergic diseases. It therefore seems likely that the increase has a multifactorial background. Based on the criteria used in this review, the rise of allergy prevalence can be considered an expected event, in perfect agreement with the predictions made by Blackley (3) almost 130 years ago.

Conclusion

The reasons for the increasing prevalence of allergic diseases over the last few decades in the Western world are unknown. It is generally believed that the causes will

be found among factors in the environment. Plausible causative environmental factors should be able to polarize the immune system toward a Th-2 dominated cytokine profile. Furthermore, the factors should be distributed in nature in such a way as to explain the reported differences in the prevalence of allergic diseases between rich and poor people, between urban and rural areas, and between Eastern and Western countries.

By these criteria, a general change in the "microbial load" seems to be the most probable cause of the increase in the allergic diseases. This conclusion is based on the known propensity of many microbes to stimulate the Th1 immune system, which suppresses Th2-type immunity; the apparently allergy-protective effect of

poor hygiene; the probable allergy-stimulating effect of early antibiotic treatment in infants; the inverse relationship between certain infections and allergy as well as between indoor air content of endotoxin and allergy; and, finally, the association between certain bacteria of the gut microflora and development of allergy. It thus seems likely that atopy is a "microbial deprivation disorder". There are, however, also other factors such as dietary components, diesel exhaust particles, and environmental toxins and pollutants that may play a causative role in the increasing prevalence of allergy. Of particular interest are changed dietary habits concerning fatty acids and antioxidants, which play important roles in the polarization of immune responses.

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